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Effect of anti-allergic compounds on anaphylactic histamine secretion from rat peritoneal mast cells in the presence and absence of exogenous calcium

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Summary. The anti-allergic drugs theophylline, doxantrazole, quercetin, 8-bromo cyclic AMP, and disodium cromoglycate prevented anaphylactic histamine release from rat peritoneal mast cells in both the presence and absence of extracellular calcium. The activity of the test compounds under the latter conditions cannot be simply explained in terms of their postulated ability to block movement of the cation from the external environment into the cell. Alternative modes of action are thus considered.

INTRODUCTION

Anaphylactic histamine release from the mast cell is triggered by an increased concentration of ionized calcium in the cytosol (Foreman, Garland & Mongar, 1976). This calcium may be derived from intra- or extracellular sources according to the conditions of the experiment. In the presence of exogenous calcium the immunological stimulus increases the permeability of the cell membrane to the cation, that is, opens calcium gates in the membrane (Foreman *et al.*, 1976). Influx of the ion from the external environment then initiates the release process. In the absence of added calcium, the stimulus mobilizes sequestered or membrane-bound stores of the cation (Ennis, Truneh, White & Pearce, 1980c; Pearce, Ennis, Truneh & White, 1981; White & Pearce, 1982). This mobilization may be facilitated by brief pretreatment of the cells with chelating agents.

In view of the central role of the ion in the release mechanism, factors which modulate calcium homeostasis can clearly regulate secretory activity. It has been suggested that a number of anti-allergic drugs may inhibit histamine release by acting directly on the gating mechanism to prevent movement of calcium from the extracellular milieu into the cytosol (Fewtrell & Gomperts, 1977; Foreman *et al.*, 1976; Foreman, Hallett & Mongar, 1977a, 1977b; Foreman, Mongar, Gomperts & Garland, 1975). To examine this hypothesis further we have investigated the ability of the test drugs to block anaphylactic histamine release in the presence and absence of added calcium.

This work complements our previous studies with basic secretagogues (Ennis, Atkinson & Pearce, 1980a; Ennis, Pearce & Weston, 1980b; Ennis & Pearce, 1979), the ionophore A23187 (Pearce & Truneh, 1981), and concanavalin A (Truneh & Pearce, 1981).

MATERIALS & METHODS

Rats were immunized with the nematode *Nippostrongylus brasiliensis* and peritoneal cells recovered as described (White & Pearce, 1982). Anaphylactic histamine secretion was similarly determined and all values

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were corrected for the spontaneous release (ca. 5%) occurring in the absence of the inducer.

The effect of various inhibitors was studied in the presence and absence of exogenous calcium (1 mM). Ethylenediamine tetraacetic acid (EDTA, 0·1 mM) and phosphatidyl serine (PS; Lipid Products, Redhill, 15 μg/ml) were added as indicated. Disodium cromoglycate (a gift from Mr P. M. Sheard, Fisons Pharmaceuticals, Loughborough) was active without preincubation and was added to the cells simultaneously with the allergen. Cells were preincubated (5 min, 37°) with doxantrazole (donated by Dr L. G. Garland, Wellcome Research Laboratories, Beckenham), theophylline and quercetin (Sigma London Chemical Co.). They were then challenged with allergen (20 WE/ml). In further experiments, cells were preincubated (30 min) with N^6-$O^{2'}$-dibutyryl adenosine-3′,5′-cyclic monophosphate (dibutyryl cyclic AMP, Bu_2cAMP) or 8-bromoadenosine-3′,5′-cyclic monophosphate (8-bromo cAMP, Sigma London Chemical Co.) in the presence of calcium to prevent depletion of intracellular stores of the ion (Ennis & Pearce, 1979). They were then recovered by centrifugation, washed and resuspended in the appropriate medium containing the cyclic nucleotide before challenge. In all experiments, unless otherwise stated, secretion was assessed 10 min after addition of the allergen. In general, results are expressed in terms of the percentage inhibition of the control release and thus given as the ratio: [(% histamine release in absence of drug − % histamine release

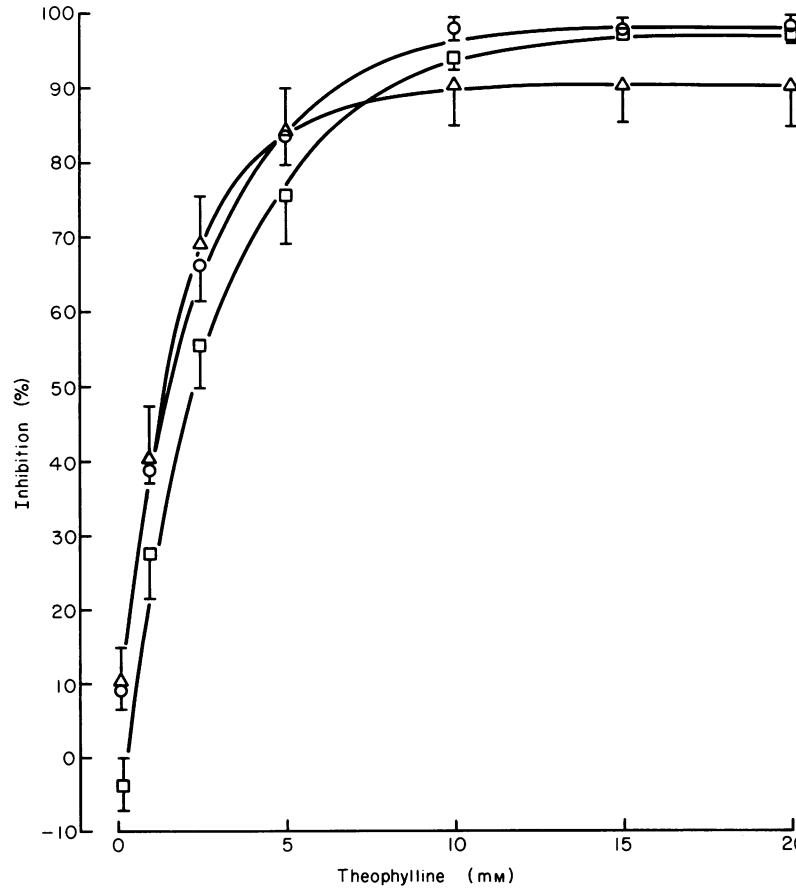

Figure 1. Effect of theophylline on anaphylactic histamine release in media containing calcium (△), no calcium (○), and EDTA (□). Values are means ± SE for four experiments and are expressed as percentage inhibition of the control releases (△ 35·8 ± 5·0, ○ 29·9 ± 5·3 and □ 32·7 ± 3·8). All experiments were carried out in the absence of PS.

in presence of drug)/(% histamine release in absence of drug)] × 100. Values are recorded as means ± SE.

RESULTS

All of the test drugs (with the exception of Bu_2cAMP, see below) produced dose-dependent inhibition of histamine release in the presence and absence of extracellular calcium. The effects of theophylline, doxantrazole, quercetin and 8-bromo cAMP were essentially independent of the ionic composition of the medium. Some representative data for theophylline in the absence of PS are shown in Fig. 1. The inhibitory effect of the drug was slightly reduced under all conditions by addition of the lipid, the ID_{50} (the dose required to effect 50% inhibition of release) increasing from 1–2 mM to 4–6 mM (data not shown). The effects of doxantrazole, quercetin and 8-bromo cAMP were essentially independent of PS and some illustrative results are shown in Figs 2 and 3 and in Table 1. Bu_2cAMP, at concentrations up to 10 mM, had a negligible effect on histamine release (data not shown). In the absence of PS, cromoglycate reduced histamine secretion in all the test media (Fig. 4a). The inhibitory potency of the compound was reduced by addition of the lipid (Fig. 4b), the effect being most marked in the presence of exogenous calcium. However, the drug was considerably more effective if cells were stimulated under these conditions for only short periods of time, the activity decaying rapidly with prolonged incubation (Table 2).

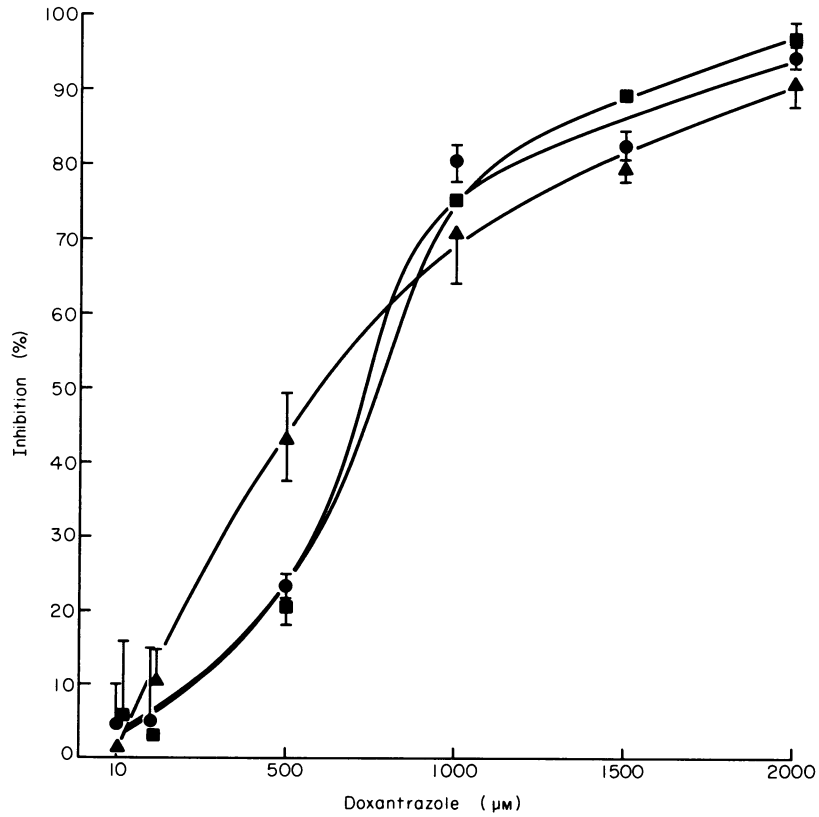

Figure 2. Effect of doxantrazole on anaphylactic histamine release in media containing calcium (▲), no calcium (●), and EDTA (■). Values are means ± SE for four experiments and are expressed as percentage inhibition of the control releases (▲ 59·5 ± 3·2, ● 17·8 ± 4·9 and ■ 39·9 ± 4·8). All experiments were carried out in the presence of PS (15 μg/ml).

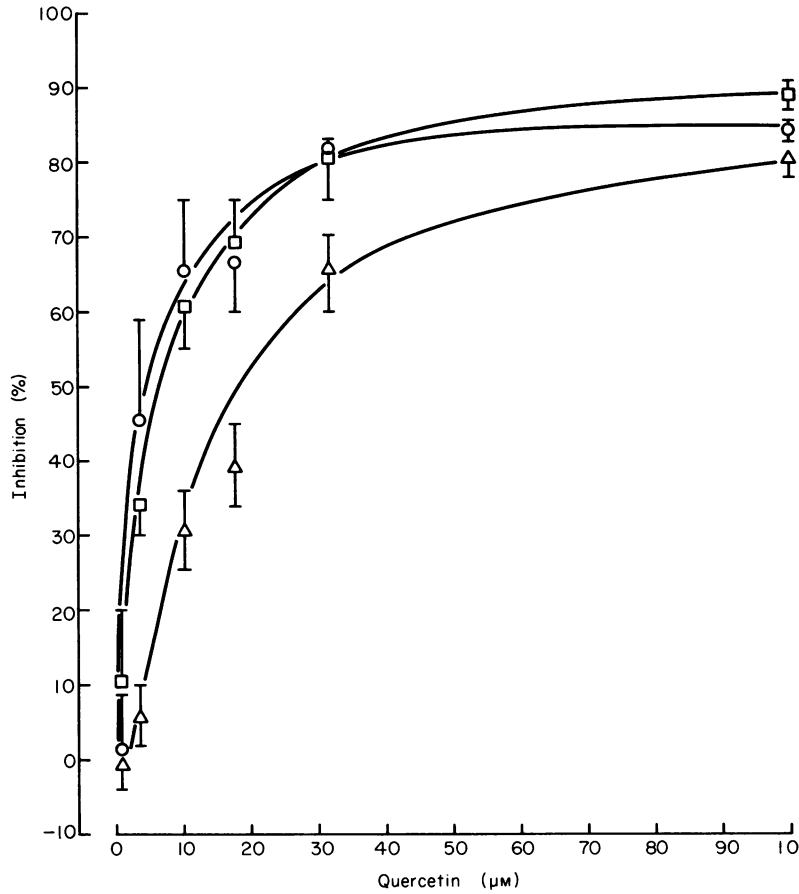

Figure 3. Effect of quercetin on anaphylactic histamine release in media containing calcium (△), no calcium (○), and EDTA (□). Values are means ± SE for four experiments and are expressed as percentage inhibition of the control releases (△ 40·8 ± 4·0, ○ 24·9 ± 7·6 and □ 43·7 ± 3·1). All experiments were carried out in the absence of PS.

Table 1. Inhibition of anaphylactic histamine release by 8-bromo cAMP

	Inhibition (%) in media containing:		
8-bromo cAMP (mM)	Calcium (1 mM)	No calcium	EDTA (0·1 mM)
10	83·1 ± 4·1	96·0 ± 3·0	96·0 ± 3·9
1	17·1 ± 8·3	23·0 ± 12·4	21·3 ± 14·3
0·1	4·6 ± 2·5	8·2 ± 8·2	3·1 ± 15·7

Cells were preincubated (30 min, 37°) with the stated concentrations of 8-bromo cAMP in the presence of calcium (to permit accumulation of the nucleotide but prevent removal of intracellular calcium), recovered by centrifugation, washed and resuspended in the media shown containing the nucleotide. They were then challenged with allergen (10 WE/ml) in the absence of PS. Values are means ± SE for three experiments and are expressed as percentage inhibition of the control releases (calcium 29·5 ± 0·9, no calcium 23·5 ± 1·2, and EDTA 31·5 ± 3·1).

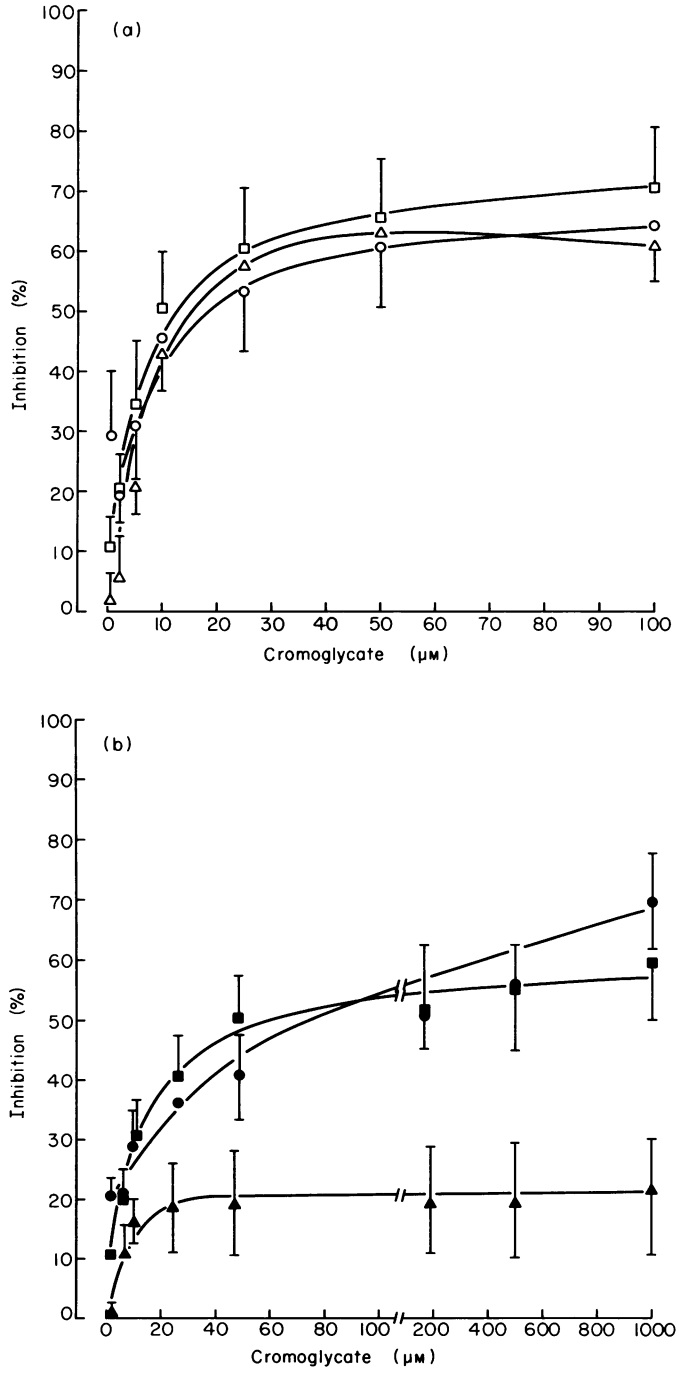

Figure 4. Effect of cromoglycate on anaphylactic histamine release in media containing calcium (△, ▲), no calcium (○, ●) and EDTA (□, ■). Open symbols denote the absence (a) and closed symbols the presence (b) of PS (15 μg/ml). Values are means ± SE for six experiments and are expressed at percentage inhibition of the control releases (△ 28·6 ± 4·8, ▲ 74·9 ± 6·1; ○ 13·6 ± 1·8, ● 25·0 ± 3·7; □ 29·3 ± 2·4, ■ 26·4 ± 2·7).

Table 2. Inhibition by cromoglycate (100 μM) of anaphylactic histamine release from cells stimulated for different times

Time (sec)	Control histamine release (%)	Inhibition (%)
10	29·0±8·2	72·5±10·3
20	44·0±6·6	60·2±13·5
30	51·3±6·5	45·7±13·3
40	57·3±4·9	36·4±11·3
60	57·7±4·4	37·4±13·0
150	59·4±4·0	25·9±9·3
300	59·9±3·9	21·4±5·5
600	60·1±3·8	20·3±5·5

Cells (1 ml) were stimulated for the periods shown with allergen (20 WE/ml) in the presence of PS (15 μg/ml) and cromoglycate (100 μM). The reaction was then terminated by the addition of ice-cold buffer (3 ml). Values are means±SE for four experiments and are given as percentage inhibition of the appropriate control releases.

DISCUSSION

Theophylline, doxantrazole, quercetin, cyclic AMP analogues and cromoglycate are claimed to prevent entry of external calcium into the mast cell and thus to block exocytosis (Fewtrell & Gomperts, 1977; Foreman *et al.*, 1975, 1976, 1977a, 1977b). However, in the present investigation these compounds each produced a comparable inhibition of anaphylactic histamine secretion in both the presence and absence of extracellular calcium.

Addition of PS enhanced the control release of histamine in the presence of calcium and markedly reduced the potency of cromoglycate under these conditions. There are a number of possible explanations of this effect. Firstly, PS might combine with and partially inactivate the compound. Such a combination would require the involvement of calcium ions since the effect was much more pronounced in the presence of the cation. Secondly, PS considerably potentiates the level of induced secretion and we, and others, have shown that the effectiveness of a given inhibitor varies markedly with the strength of the releasing stimulus (Ennis & Pearce, 1979; Ennis *et al.*, 1980a, 1980b). Finally, antigen and PS provide a particularly persistent signal for histamine release (White & Pearce, 1982). The activated state induced by antigen alone is transient and decays rapidly with time. This decay is largely abrogated by PS (White & Pearce, 1982). In order for its inhibitory action to be observed, cromoglycate then has to prevent anaphylactic histamine release only during the brief period immediately following stimulation and before the natural decay of the activated state, but has to block release induced in the presence of PS during the entire period of incubation. This effect may be particularly important for the cromone which is active only if added at the time of challenge, the inhibitory response falling off rapidly with preincubation (Thomson & Evans, 1973). Consistently, cromoglycate effectively prevented histamine secretion evoked in the presence of PS if the cells were stimulated for only very short periods of time. These conditions may approximate more closely to the transient signal provided by antigen alone.

Clearly, the activity of the test drugs in the absence of extracellular calcium cannot be simply explained in terms of their ability to block movement of the ion from the external environment into the cell. However, the location of the internal stores of calcium utilized by the anaphylactic reaction is not known and the mechanisms of release in the presence and absence of the added cation may be related. Activation of the mast cell membrane may involve the opening of a gated channel which normally contains appreciable amounts of bound calcium. Extracellular calcium (when present) would then enter the channel, displace the bound ion into the cytosol and so trigger secretion. In the absence of added calcium, the immunological stimulus could displace the ion directly by local perturbation of the cell membrane. However, even if this mechanism were correct, inhibitory compounds would have to prevent efflux of calcium from the channel into the cytosol rather than the gated influx into the channel from the extracellular environment. In the absence of extracellular calcium these events are not causally related. Moreover, this mechanism is quite inconsistent with the inhibitory effects of the test drugs on histamine release induced by the calcium ionophore A23187 (Pearce & Truneh, 1981). This compound completely circumvents the calcium gating mechanism (Foreman *et al.*, 1977a). Other possible activities for the compounds should then be considered. The agents might activate appropriate pumps to extrude calcium from the cytosol or to promote sequestration of the ion into internal stores (Berridge, 1975). Alternatively, the drugs might have effects less immediately related to calcium homeostasis and affect the organization of the structural and cytoskeletal elements involved in exocytosis (Gillespie & Lichtenstein, 1972), promote the regulatory phosphorylation of proteins in the plasma or perigranular membranes

(Theoharides, Sieghart, Greengard & Douglas, 1980), or have an as yet ill-defined stabilizing effect on the mast cell membrane. Work is in progress to distinguish between these possibilities.

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